

Pathogen inoculation procedure

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 An abbreviated version of this protocol was published in eLIFE in Jun 2015

Negative regulation of ABA signaling by WRKY33 is critical for *Arabidopsis* immunity towards *Botrytis cinerea* 2100

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Detailed protocol

Protocol for *Botrytis cinerea* infection

Prepare the *B. cinerea* spores:

The mycelium of *B. cinerea* was grown on PDA medium in the Petri Dish for about 10 days (5 to 10 plates each time).

Add the sterile water or 0.8% NaCl solution (10 ml for each plate) in the Petri Dish and wash the mycelium with a glass pipet to harvest the *B. cinerea* spores.

Collect all the mycelium solution in a falcon 50 ml or a sterile bottle 200ml and mix vigorously.

Filter the mycelium solution with three layer sterile gauze in a new clean falcon or bottle.

Centrifuge 10 min, 2000 rpm, 4 °C and remove the supernatant.

Resuspend the spores pellet with sterile water and dilute to the final concentration 2×10^7 spores/ml and stock at -80°C.

For the *B. cinerea* infection:

Grow *Arabidopsis* plants in a clean microbe-free climate chamber under the short day condition for about 4 weeks.

Prepare a solution of 2.5×10^5 spores/ml in the Vogel buffer (1 L: 15 g of Sucrose, 3 g of Na-citrate, 5 g of K_2HPO_4 , 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of $CaCl_2 \cdot 2H_2O$, and 2 g of NH_4NO_3) from the solution stock.

Infect the intact plants with two droplets of 2 µl each of this solution (on one leaf surface). For mock treatment, Vogel buffer alone was used.

Put all the plants in sealed hoods to keep high humidity and photo plants until 3 days.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Liu, S. (2020). Pathogen inoculation procedure. Bio-protocol Preprint. bio-protocol.org/prep580.
2. Liu, S., Kracher, B., Ziegler, J., Birkenbihl, R. P. and Somssich, I. E. (2015). Negative regulation of ABA signaling by WRKY33 is critical for *Arabidopsis* immunity towards *Botrytis cinerea* 2100. eLIFE. DOI: [10.7554/eLife.07295](https://doi.org/10.7554/eLife.07295)

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